

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>BP110046/KET</b>	<b>FOR FURTHER ACTION</b>		See Form PCT/IPEA/416
International application No. <b>PCT/FI2004/000654</b>	International filing date ( <i>day/month/year</i> ) <b>05.11.2004</b>	Priority date ( <i>day/month/year</i> ) <b>06.11.2003</b>	
International Patent Classification (IPC) or national classification and IPC <b>D21H21/04</b>			
Applicant <b>KEMIRA OYJ et al.</b>			
1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of 5 sheets, including this cover sheet. 3. This report is also accompanied by ANNEXES, comprising: a. <input checked="" type="checkbox"/> <i>sent to the applicant and to the International Bureau</i> a total of 4 sheets, as follows: <input type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions). <input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box. b. <input type="checkbox"/> ( <i>sent to the International Bureau only</i> ) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).			
4. This report contains indications relating to the following items: <div style="display: flex; justify-content: space-between;"> <div style="width: 15%;"> <input checked="" type="checkbox"/> Box No. I  <input type="checkbox"/> Box No. II  <input type="checkbox"/> Box No. III  <input type="checkbox"/> Box No. IV  <input checked="" type="checkbox"/> Box No. V  <input type="checkbox"/> Box No. VI  <input type="checkbox"/> Box No. VII  <input checked="" type="checkbox"/> Box No. VIII           </div> <div style="width: 85%;">             Basis of the opinion              Priority              Non-establishment of opinion with regard to novelty, inventive step and industrial applicability              Lack of unity of invention              Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement              Certain documents cited              Certain defects in the international application              Certain observations on the international application           </div> </div>			
Date of submission of the demand  <b>06.09.2005</b>	Date of completion of this report  <b>21.03.2006</b>		
Name and mailing address of the international preliminary examining authority:  <div style="display: flex; align-items: center;"> <div>             European Patent Office              D-80298 Munich              Tel. +49 89 2399 - 0 Tx: 523656 epmu d              Fax: +49 89 2399 - 4465           </div> </div>	Authorized Officer  <b>Karlsson, L</b>  Telephone No. +49 89 2399-8424		

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# INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.  
PCT/FI2004/000654

## Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
  - ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
    - ☐ international search (under Rules 12.3 and 23.1(b))
    - ☐ publication of the international application (under Rule 12.4)
    - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements\*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

### Description, Pages

1-13 as originally filed

### Claims, Numbers

1-14 received on 06.09.2005 with letter of 06.09.2005

### Drawings, Sheets

1/1 as originally filed

- ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing
3. ☐ The amendments have resulted in the cancellation of:
    - ☐ the description, pages
    - ☐ the claims, Nos.
    - ☐ the drawings, sheets/figs
    - ☐ the sequence listing (*specify*):
    - ☐ any table(s) related to sequence listing (*specify*):
  4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
    - ☐ the description, pages
    - ☐ the claims, Nos.
    - ☐ the drawings, sheets/figs
    - ☐ the sequence listing (*specify*):
    - ☐ any table(s) related to sequence listing (*specify*):

\* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT  
ON PATENTABILITY**

International application No.  
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**Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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1. Statement

Novelty (N)	Yes: Claims	1-14
	No: Claims	
Inventive step (IS)	Yes: Claims	1-10
	No: Claims	11-14
Industrial applicability (IA)	Yes: Claims	1-14
	No: Claims	

2. Citations and explanations (Rule 70.7):

**see separate sheet**

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**Box No. VIII Certain observations on the international application**

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The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**Re Item V.**

1.1 The following documents are referred to in this communication:

D1 : US 5 349 874 A (SCHAPIRA ET AL) 27 September 1994 (1994-09-27)

D2 : EP 1 350 431 A (KURITA WATER INDUSTRIES LTD) 8 October 2003 (2003-10-08)

1.2 The present invention refers to a method for detecting the presence of biofilm-forming microorganisms in a paper or board making process for determining the need of an anti-biofilm agent. The process is defined by the process steps (a)-(d) of claim 1. The assembly kit of claim 11 is defined by features (i),(iii),(iv) and (vi).

1.3 D1 and D2 disclose a method for monitoring a biofilm formation, wherein a sampler is used to collect a biofilm, which may be further cultured and analysed (see D1, claims 1-28, example 1; see D2, claims 1-24, examples 1-9). The specific process features of the present claim 1 cannot be seen in D1 and D2. Thus, it seems as the subject-matter of satisfies the requirements of Article 33.2 PCT.

Furthermore, there are no incentives to be found anywhere in D1 or D2, which would lead the skilled person to modify the processes of D1 or D2 in order to arrive at the defined combination of process features of the present claim 1. Hence, claim 1 also meets the requirements of Article 33.3 PCT.

1.4 For the reasons given above, the dependent claims 2 to 10 are also considered to meet the requirements of Article 33.2 and 33.3 PCT.

1.5 As already explained above in item 1.3, it is known from D1 and D2 to apply a sampler to obtain a biofilm, which will be cultured and, somehow, detected. The skilled person would from D3:EP-A-1 118 859 and D4:WO-A-99 065 89 get the teaching that the culturing of the biofilm may be carried out on a plate which is provided a plurality of recessions for the sampler devices (see D3, paragraphs [0167],[0196], claims 1-38; see D4, clams 1-10, figure 1). It is further revealed in the above documents that the culturing plate may be agitated. Hence, it seems that the combination of the disclosure of D1 or D2 with the teaching of D3 or D4 would lead the skilled man to the subject-matter of the

clearly defined features of claim 11 (Art.33.3 PCT).

1.6 The rest of the separate features of the dependent claims 12 to 14 do not seem to contain any novel and inventive features with regard to the disclosures of D1,D2,D3 and/or D4 (Art.33.2 and 33.3 PCT). However, a combination of these features, especially a clarified claim 1, may nevertheless satisfy the requirements of Article 33.3 PCT.

**Re Item VIII.**

2.1 According to the description is one of the objects of the present invention to enable a more timesaving method for monitoring the microbiological state of the paper making process. However, the wording "for a period of time" does by no means restrict the time necessary for forming the in situ film of microorganisms. Thus, it seems as the essential features of the present invention is missing in claim 1 (Art.6 PCT).

The corresponding remarks also applies to process feature (c). Besides, it appears as the very generally defined culturing device of feature (c) would embrace also a conventionally Agar plate (Art.6 PCT).

2.2 Process feature (d) of claim 1 has neither been clearly defined, since according to the description, especially the detection operation should distinguish the claimed invention from the prior art. This feature of claim 1 does only very generally define that it should somehow be detected, qualitatively or quantitatively, the presence or absence of a biofilm. In the broadest sense this would mean that the recesses of the culturing device is examined by, e.g. the paper maker (cf claim 11).

It seems as the necessary features for detecting the presence or absence of a biofilm are lacking in claims 1 and 11.

2.3 Presently there seems to be an inconsistency between the features of claims 1 and claim 11, since features (ii) and (v) are still not clearly defined in claim 11 (Art.6 PCT). Besides, claim 11 refers to three different units, i.e. the sampler, a plate and the reagents.

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**Claims**

1. A method for detecting the presence of biofilm-forming microorganisms in a paper or board making process for determining the need of an anti-biofilm agent in the process, **characterized by the steps comprising:**
  - 5 (a) subjecting a sampler device in the process line for a period of time to enable said microorganisms to form a biofilm *in situ* in said process on the surface of the sampler,
  - (b) treating the surface of the sampler with said formed biofilm thereon in a solution of a test anti-biofilm agent for a period of time, then
  - 10 (c) contacting the surface of the sampler with said biofilm thereon with a liquid growth medium in a recession of a culturing device for a period of time,
  - (d) removing the growth solution and the surface of the sampler from the recession of said device and detecting qualitatively and/or quantitatively the presence or absence of biofilm-forming microorganisms adhered on the walls
  - 15 of the recession.
2. The method according to claim 1 or 2, **characterized** after the biofilm formation said surface of the sampler is (b) treated with the solution of the test anti-biofilm agent for the selection of the most efficient anti-biofilm agent.
3. The method according to any of the preceding claims, **characterized** in that
  - 20 (a) subjecting a sampler device in the process line for a period of 12 h to 3 d,
  - (b) effecting the optional treatment step with the solution of a test anti-biofilm agent for a period of e.g. 10 minutes to 4 hours, preferably for 1-2 h, between the ambient temperature and 65°C, preferably at the temperature close to the process temperature of the sampling site of the process line, such as at 40-
  - 25 60°C, then
  - (c) effecting the culturing step, preferably with shaking, in a liquid growth medium in a recession of a culturing device for a period of e.g. for 8-48 h, preferably for 8-24 h, at the temperature between the ambient temperature and 65°C, e.g. at 35-65°C, preferably close to the process temperature of the sampling
  - 30 site of the process line, such as at 40-60°C.

4. The method according to any of the preceding claims, characterized in that (b) the treatment is effected in a treatment device provided with a recession which is filled with a solution comprising the test anti-biofilm agent and a liquid growth medium, sterilized water and/or process water by immersing said surface of the sampler in said solution.
5. The method according to any of the preceding claims, characterized in that the step (c) is effected in a culturing device provided with a recession which is filled with the liquid growth medium by immersing said surface of the sampler in said solution.
6. The method according to any of the preceding claims, characterized in that (d) the sampler surface and the growth solution is removed from the recession of the culturing device, the recession is optionally washed and any biofilm-forming microorganisms adhered on the walls of the recession are stained and the presence and/or intensity of the color formation in the recession is detected qualitatively or quantitatively.
7. The method according to any of the preceding claims, characterized in that (a) the sampler device comprises a plurality of elongated protrusions connected to a support, whereby, when brought in the process, the biofilm is formed on the surface of the protrusions.
8. The method according to claim 8, characterized in that (b) the treatment device is provided with a plurality of recessions containing a solution comprising one or more test anti-biofilm agents in one or more concentrations, one test anti-biofilm agent at one concentration in each recession, and said solution without any test anti-biofilm agent as a reference, and that the protrusions of said sampler removed from the process line are immersed in said solution in the recessions, one protrusion in each recession.
9. The method according to claim 8 or 9, characterized in that (c) the culturing device comprises a plurality of recessions containing the liquid growth medium, and that the protrusions of said sampler, optionally treated in step (b), are immersed in said growth solution in the recessions of the culturing device, one protrusion in each recession.
10. The method according to any of the preceding claims, characterized in that the sampler device comprises a plurality of pins or pegs arranged in rows and fixed from one end on a support plate and the treatment device of the optional step

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(b) and the culturing device of the step (c) are multi-well plates provided with a plurality of wells arranged in rows and adapted for receiving one protruding pin in one well so that each pin of the sampler device sits in each well of the plate of the treatment and culturing device.

- 5 11. An assembly kit for detecting the presence of biofilm-forming microorganisms according to the method of claim 1 in a paper-making or board-making process, and for determining the need of an anti-biofilm agent in the process, and optionally for the selection of the most efficient anti-biofilm agent, comprising at least a combination of
- 10 (i) a sampler device comprising a plurality of elongated protrusions connected to a support for enabling said microorganisms to form a biofilm *in situ* in said process on the surface of the sampler,
- (ii) an optional treatment device comprising a plate provided with a plurality of recessions arranged to receive one protrusion of the sampler device in each
- 15 recession thereof,
- (iii) a culturing device comprising a plate provided with a plurality of recessions arranged to receive one protrusion of the sampler device in each recession thereof,
- (iv) a shaker for shaking the treatment and/or the culturing device, and
- 20 (v) optionally a detector for detecting the presence or absence of any biofilm-forming microorganisms adhered in the recessions of the culturing device,
- (vi) reagents comprising
- (a) one or more test anti-biofilm agents suitable for the paper industry, preferably in one or more dilutions,
- 25 (b) a liquid growth medium,
- (c) staining agent, and
- (d) optionally a washing solution.

12. The assembly kit according to claim 12, characterized in that it comprises (i) a sampler provided with a plurality of pins in rows fixed from one end on a lid and
- 30 (ii) the treatment device and (iii) the culturing device, which are both multi-well



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plates provided with a plurality of wells in rows, whereby the pins of the sampler sit in the wells of the treatment and of the culturing plate, one pin in each well, when the sampler lid is placed on the treatment or the culturing plate.

- 5 13. The assembly kit according to claim 12 or 13, characterized in that (ii) the wells of the treatment plate are filled with a solution of one or more test anti-biofilm agents, at one or more concentrations, in a liquid growth medium or sterilized water, one test anti-biofilm agent in each well, and that at least one well is filled with a growth medium or sterilized water alone, and (iii) the wells of the culturing plate are filled with a liquid growth medium, and that the wells are sealed off with a  
10 removable cover.

14. The use of the detection method of any of claims 1-11 or of the assembly kit of any of claims 12-14 for monitoring the paper or board-making process to determine the need of an anti-biofilm agent in the process, and for the selection of the most efficient anti-biofilm agent.